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(54) Title: ADDUCTS OF QUINOLONIC AGENTS WITH NATURAL POLYSACCHARIDE POLYMERS

(57) Abstract: Adducts of natural polyssacharide polymers with quinolonic antibacterial agents, in the form of aqueous solutions or solid powder form, possess an identical therapeutic efficacy if compared with the corresponding active ingredient alone, used at the same doses. Therefore they show lower toxicity with equal antimicrobial activity.

The adducts according to the present invention are in powder form or in the form of an aqueous solution that preferably contains them in a concentration of between 0.1 and 1% by weight calculated on the total weight of the aqueous solution.

The present invention further relates to the process for preparing the aforesaid adducts which, in particular, comprises the following steps:

a) preparing the solution of the polysaccharide polymer in water in a percentage of ingredient of between 20 and 60% by weight on the total weight of the adduct and adding the active ingredient, thereby obtaining a concentration of the adduct being preferably comprised in the aforesaid intervals,

b) filtering the aqueous solution thus obtained, in case the adduct in aqueous solution form is achieved,

c) removing water thereby obtaining the adduct according to the present invention in powder form.

The present invention further relates to pharmaceutical compositions containing the adducts according to the present invention in association with suitable excipients and/or diluents.

DETAILED DESCRIPTION OF THE INVENTION

The present invention represents an optimum form for administering both orally and parenterally quinolonic antibacterial drugs to be used in the treatment of infections caused by Gram-negative and Gram-positive bacteria.

More specifically, the present invention concerns adducts between an active ingredient, belonging to the class of Quinolones, and a natural biocompatible polysaccharide, able to interact without forming covalent and ionic bonds.

The adducts can be administered both orally and parenterally, depending on the physical-chemical characteristics and the pharmacodynamic and pharmacokinetic profile of the active ingredient.

The active ingredients used in the adducts according to the present invention are preferably nalidixic acid, pipemidic acid, cinoxacin, norfloxacin, cyprofloxacin, pefloxacin, enoxacin, ofloxacin and the levo form thereof levofloxacin. These are mainly used in the treatment of infections caused by Gram-negative and positive bacteria and their action mechanism consists in blocking DNA synthesis by inhibiting the DNA enzyme gyrase.

The selected natural polysaccharides are biocompatible and inert and therefore their quinolones adducts do not present problems with quinolones insofar as it concerns toxicity and also from an immunological stand point.

The preparation of the adducts envisages the solution of the polymer in water, in a percentage that may vary between 20 and 60% in weight, and the subsequent addition of the active ingredient, allowing the hydrophilic interaction of carboxylic and aminic groups of the antibacterial agent with those of the polymer. Weak hydrogen type bonds are thus formed with lower interaction forces if compared to covalent or ionic type bonds (Remington's Pharmaceutical Science 18th ed. p. 186).

The polymer and the drug are dissolved in distilled water (optionally with buffers and preservatives), filtering is performed to obtain a clear solution. Conversely for preparing the adduct in solid form, the solvent is removed from the adduct through a process of freeze-drying or nebulization (spray drying) thereby obtaining the adduct in solid form.

For the preparation of injectable forms, depyrogenated and sterile (w.f.p.) distilled water is used and the solution is filtered, with filters of from 0.1 to 0.2 μm porosity, then placed in depyrogenated and sterilized phialoids in a sterile environment, and preferably freeze-dried. The solution can be readily reconstituted by adding an aqueous solvent such as w.f.p. water or a physiological solution.

Distilled water is used for the preparation of oral forms and the solution is filtered on filters of 0.45 μm porosity. The solvent is removed preferably through nebulization, and a solid porous adduct is obtained suitable for the preparation of pharmaceutical forms for oral use, such as tablets, capsules and granules.

The titre of the active ingredient in the adduct was detected by HPLC, using a Perkin-Elmer LC75 chromatograph with an LC Pump 414-T Karitron Analytical injector, Waters detector-integrator and a Lichrosorb column RP18.

The in vitro microbiological activity of these adducts compared to that of the corresponding quinolones was determined both by the measurement of inhibition halos developed on plate as well as by the detection of the minimum concentration able to inhibit the bacterial growth in the sample tube.

The in vivo activity was assessed in mice previously infected with a strain which is

after being filtered on a filter with a porosity of 0.45 µm, is spray dried with a mini spray drier (Mini Buchi). The ejecting pressure is 800 mbar, the inlet temperature is 130°C and outlet temperature is 50°C and the suction is maintained at the maximum of capacity.

1.51 g of ivory coloured adduct are obtained in granular powder form, soluble in water and with a norfloxacin titre of 59.5% by weight. With the addition of suitable excipients the adduct is suitable to be formulated in capsules, sachets, and after being compressed in tablets.

EXAMPLE 2 - Preparation of the solid adduct: 50% dextran - 50% norfloxacin

1 g of dextran 5 is dissolved in 1 L of distilled water and 1 g of norfloxacin is added to the solution, bringing the pH to the value of 4.8 by the addition of 1N HCl. The solution is filtered with a filter of 0.45µm porosity and the solvent is removed by spraydrying as described in example 1. 1.87 g of whitish coloured adduct are obtained in the form of a light powder, with a norfloxacin titre of 49% in weight. The powder added with suitable excipients can be placed in sachets or formulated into capsules or directly compressed.

EXAMPLE 3 - Preparation of the solid adduct: 50% maltodextrin - 50% cyprofloxacin

2 g of 500M maltodextrin are solubilized in 1 L of distilled water under magnetic stirring.. 2.5 g of cyprofloxacin lactate are added (corresponding to 2 g of active ingredient) while maintaining under stirring and adding 1N HCl up to total solubilization. The solution is filtered and spraydried following the same conditions of the previous example.

4.2 g of ivory coloured adduct are obtained as a light powder, with a cyprofloxacin titre of 49.7% by weight. The powder added with suitable excipients can be placed into sachets or formulated into capsules or directly compressed.

EXAMPLE 4 - PREPARATION OF THE ADDUCT: 40% INULIN - 60% CYPROFLOXACIN

1.6 g of inulin are dissolved under magnetic stirring in 800 ml of w.f.p. water and added with 3 g of cyprofloxacin lactate (2.4 g cyprofloxacin) adding of 1N HCl up to complete solubilization. Additional w.f.p. water is added to adjust the total volume to 1 L.

Dissolving the contents of each vial in 15 ml of a solvent based on a physiological solution and 40 mg of inulin the extemporaneous adduct solution is reconstituted.

EXAMPLE 8 - Determination of the inhibition halos

As for the tests of in vitro antimicrobial activity of norfloxacin and cyprofloxacin, the inhibition halos were obtained with the diffusion method (FUI X ed., p. 135), using a Mueller-Hinton Medium Difco culture medium (with low thymine and thymidine content) conforming to the MG-P standards of the NCCLS in 9 cm diameter sterile Petri dishes and Tryptic soy broth (Difco).

Small discs of filter paper were prepared for the sensitivity test, by soaking them in a quinolonic antibacterial agent at defined concentrations, and the corresponding adduct small discs were prepared, in which the total dose is the same, but the active ingredient content by weight is lower.

To produce the bacterial growth the following were used: 1 strain of Staphylococcus aureus ATCC 6538 P (strain A) and 2 field strains of Staphylococcus aureus (strain B and C).

Broth cultures were prepared placing a bacterial patina loop of a culture in agar of 12 h in a test tube containing 5 ml of Sterile Tryptic Soy Broth and then incubated at 37° C for 4 h.

If necessary, the suspensions were diluted with sterile water to obtain a similar turbidity to the reference standard (0.5 Mc Farland). 150 millilitres of Mueller-Hinton Medium were sterilized in autoclave at 121°C for 15,' a part was placed of in sterile Petri plates of 9 cm diameter and left to solidify, a part was cooled to ≅ 45°C and inoculated with 1.5 ml of the bacterial suspensions previously prepared and poured on the surface of the solidified agar in a ratio of 10 ml per plate.

The sown plates were kept open to dry under a laminar flow hood for 15' and, after such time, the discs were deposited on the surface of the agar with fine pointed tweezers, previously flame sterilized and cooled. 4 discs were placed in each plate: the adduct and the quinolonic antibacterial agent at the two concentrations that were to be compared. The plates thus prepared, 5 for each test, were incubated upside down at 37°C for 16 h and after this time the inhibition halos were calculated.

The results obtained are contained in Table I

EXAMPLE 10 - In vivo activity

Solutions of the two antibacterial agents and the respective adducts ay various dilutions with purified water for oral use were administered to common albino mice each weighing 28 g, every 24 h for five days. They had previously been infected with Staphylococcus aureus (that did not show resistance to the antibacterial agents under consideration), and afterwards they had therefore developed an ulcerative dermatitis with localized abscesses.

After the treatment a bacteriological control was carried out by reisolation, taking some skin swabs of the animals and proceeding with the plate inoculum of the material taken. The results are contained in tables III and IV.

Table III

Compound	N° infected subjects	Post infection clinical manifesta-tions	Bacteriological examination after 5 days of therapy		Recovery %
			Positive	Negative	
Norfloxacin	15	yes	1	14	93.3%
Adduct of example 2	14 1	Yes No	2 0	12 0	85.7%
Infected control	15	Yes	15	0	
Non-infected control	15	no			

CLAIMS

1. Adduct of a quinolonic type antibacterial agent with a polysaccharide polymer of natural origin, characterized in that the percentage of said active ingredient in the adduct is comprised between 40 and 80% by weight out calculated on the total weight of the adduct.

2. Adduct according to claim 1 wherein it is in the form of an aqueous solution.

3. Adduct according to claim 2 wherein it is contained in said aqueous solution in concentrations of between 0.1 and 1% by weight calculated on the total weight of said aqueous solution.

4. Adduct according to claim 3, wherein it is in powder form.

5. Adduct according to claim 4 wherein it is obtained by spraydrying or freeze-drying the aqueous solution according to claims 2 and 3.

6. Adduct according to any one of claims 1-5, wherein the content of quinolonic type antibacterial agent is equal to 60% by weight calculated on the total weight of the adduct.

7. Adduct according to any one of claims 1-6, wherein the quinolonic type antibacterial agent is selected from the group consisting of nalidixic acid, pipemidic acid, cinoxacin, norfloxacin, cyprofloxacin, pefloxacin, enoxacin, ofloxacin, levofloxacin.

8. Adduct according to any one of claims 1-7, wherein said polysaccharide polymers of natural type are selected from the group consisting of: dextrans, inulin, maltodextrins of pharmaceutical grade.

9. Adduct according to claim 8, wherein dextrans from 4 to 70 are used.

10. Process for preparing the adduct according to any one of claims 1-9, comprising the following steps:

a) preparing a solution of the polysaccharide polymer in water in a percentage of between 20 and 60% by weight on the total weight of the adduct, and adding the active ingredient,

b) filtering the aqueous solution thus obtained for preparing the adduct in aqueous solution form according to claim 2 or 3;

c) removing water thereby obtaining the adduct in powder form according to claim 4.

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO 00 78287 A (ANZAGHI PIERGIORGIO ;STEFLI ROSANNA (IT); ISTITUTO BIOCHIMICO PAVE) 28 December 2000 (2000-12-28) page 3, line 6 - line 8 page 3, line 18 -page 4, line 14 page 5 -page 6; examples 1,2 claims 1-14	1-18

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